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An extended sequence specificity for UV-induced DNA damage

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ABSTRACT

The sequence specificity of UV-induced DNA damage was determined with a higher precision and accuracy than previously reported. UV light induces two major damage adducts: cyclobutane pyrimidine dimers (CPDs) and pyrimidine(6–4)pyrimidone photoproducts (6–4PPs). Employing capillary electrophoresis with laser-induced fluorescence and taking advantages of the distinct properties of the CPDs and 6–4PPs, we studied the sequence specificity of UV-induced DNA damage in a purified DNA sequence using two approaches: end-labelling and a polymerase stop/linear amplification assay. A mitochondrial DNA sequence that contained a random nucleotide composition was employed as the target DNA sequence. With previous methodology, the UV sequence specificity was determined at a dinucleotide or trinucleotide level; however, in this paper, we have extended the UV sequence specificity to a hexanucleotide level. With the end-labelling technique (for 6–4PPs), the consensus sequence was found to be 5′-GCTC*AC (where C* is the breakage site); while with the linear amplification procedure, it was 5′-TCTT*AC. With end-labelling, the dinucleotide frequency of occurrence was highest for 5′-TC*, 5′-TT* and 5′-CC*; whereas it was 5′-TT* for linear amplification. The influence of neighbouring nucleotides on the degree of UV-induced DNA damage was also examined. The core sequences consisted of pyrimidine nucleotides 5′-CTC* and 5′-CTT* while an A at position "1" and C at position "2" enhanced UV-induced DNA damage.

1. Introduction

Ultraviolet light (UV) has a mutagenic and carcinogenic effect on organisms [1,2]. UV light triggers adduct formation between two adjacent pyrimidine bases along the DNA template [3]. Non-repaired adducts contribute to a genetic instability within the genome particularly in human epidermal cells, that can lead to melanoma and non-melanoma skin cancers. The genetic instability is introduced by the accumulation of UV-signature mutations, UV-caused defective DNA replication and DNA double-strand breaks [4–7].

The two major UV-induced adducts are cyclobutane pyrimidine dimers (CPDs) and pyrimidine(6–4)pyrimidone photoproducts (6–4PPs) [4]. CPDs are frequently formed at TT and TC, less often at CT and CC sequences. In contrast, 6–4PPs are preferentially present at TC, and to a lesser extent at CC and TT sequences [8–11]. While CPDs are stable, 6–4PPs are alkali-labile [12]. On treatment with high temperature and high pH, 6–4PPs are converted to DNA single-strand breaks at the pyrimidine nucleotide 3′- to the dimer [10]. The breakage products bear 3′- and 5′-phosphate termini. In addition, CPDs can also be recognised and cleaved by *Micrococcus luteus* endonuclease or T4 endonuclease V [13,14]. Antibodies that are specific for CPDs and 6–4PPs can be employed to recognise as well as isolate each type of adduct [12,15–17]. The use of antibodies in combination with the enzymes, *M. luteus* endonuclease or T4 endonuclease V, has enabled individual adducts to be selectively studied with regard to their properties, mutational profiles, distributions as well as sequence specificities [9,13,18].

UV-induced adducts can be repaired via several different pathways [5,19,20]. Photolesions that have escaped the repair system block DNA replication [21–23]. Elongation of the new DNA strand is prematurely terminated as DNA polymerases cannot bypass the adduct sites [24–26]. The cytosine base in UV-induced adducts is chemically unstable [27] and the base undergoes deamination to become a uracil base in the dimer [28,29]. As a result, UV-signature mutations of C → T are established [30–32].

Previous studies have shown that UV adducts are not randomly distributed but are preferentially formed at consecutive pyrimidine nucleotides [8,33–35]. The impact on cells can be crucial if these UV-damaged sites result in mutations in critical sections of genes [36,37]. It is important to investigate the distribution of pre-mutagenic UV adducts as they can correlate with the mutational profiles in the genome [8,11,38,39]. Sites containing 5′-TC have been reported to be UV mutational hotspots since both CPDs and 6–4PPs have a high possibility to be produced at these sites [11,38]. The UV adduct formation is purely dependent on the photon energy absorption by the DNA bases.

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Neighbouring nucleotides that surround the main adduct sites can have an enhancing or quenching effect on adduct formation and hence neighbouring nucleotides play a critical role in defining the UV damage sequence specificity [9,18,36].

Employing capillary electrophoresis with laser-induced fluorescence (CE-LIF) and taking advantages of the distinct properties of the CPDs and 6–4PPs, we investigated the DNA sequence specificity of UV light in a purified DNA sequence using two approaches: end-labelling and a polymerase stop/linear amplification assay [40–44]. The susceptibility of 6–4PPs to strand scission enables an investigation of their sequence specificity in purified fluorescently end-labelled DNA sequences. The inhibition of *Taq* DNA polymerase by both CPDs and 6–4PPs permits the determination of the sequence specificity of these adducts by the polymerase stop/linear amplification assay. Both the end-labelling and polymerase stop/linear amplification procedures were investigated utilising CE-LIF that enabled quantitative analysis at base pair resolution [42,44].

A mitochondrial DNA sequence, Mito 15, that contained a random nucleotide composition, was employed as the target DNA sequence in this study [44]. The end-labelling and the polymerase stop/linear amplification procedures were independently conducted on the same DNA sequence, Mito 15, to investigate the sequence specificity of UV-induced DNA damage. With the end-labelling method, the sequence was fluorescently end-labelled, damaged by UV and analysed by CE-LIF to determine the DNA sequence specificity [44]. For the polymerase stop/linear amplification, the plasmid was UV-exposed, linear-amplified with a single fluorescently end-labelled primer, and analysed by CE-LIF. We compared the UV damage patterns and the sequence specificities using the two approaches. Using modern technology, we determined the UV DNA sequence specificity at a longer consensus sequence than previously studied - at the hexanucleotide rather than the dinucleotide or trinucleotide level.

2. Materials and Methods

The Mito 15 sequence is shown in Fig. 1 [44] and was utilised to investigate the DNA sequence specificity of UV-induced DNA damage. The Mito 15 sequence is derived from the human mitochondrial genome and has a random nucleotide composition, that enabled the sequence specificity of UV-induced DNA damage to be determined without sequence bias. Oligonucleotides were purchased from Invitrogen/Life Technologies and the deoxynucleotide triphosphates (dNTPs) were from Thermo Fisher Scientific.

2.1. 5′-End-labelling

The 5′-fluorescent end-labelling procedure was performed as previously described [44]. For the top strand, the oligonucleotide primers were 5′-FAM-ATGTGCTGCAAGGCGA-3′ (FAM-Seq2) and 5′-ATTGTGA GCGGATAAC-3′ (Rev2); and for the bottom strand, 5′-FAM-ATTGTGA GCGGATAAC-3′ (FAM-Rev2) and 5′-ATGTGCTGCAAGGCGA-3′ (Seq2). In the 20 µl PCR reaction there was 6 ng Mito 15 plasmid DNA, 10 pmol of each oligonucleotide primer, 0.3 mM dNTPs, 16.6 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl (pH 8.8), 6.7 mM $MgCl_2$ and 0.1 U *Taq* DNA polymerase (Thermo Fisher Scientific). The thermal cycling conditions involved 5 min at 95 °C; followed by 25 cycles of 95 °C for 45 s, 55 °C for 1 min, 72 °C for 2 min; a single cycle of 72 °C for 10 min; and a final hold at 4 °C.

The labelled mitochondrial fragments were purified on a 6% (*w/v*) native polyacrylamide gel. The gel slice containing the fluorescently-labelled fragments was excised and transferred to a new tube containing 500 µl 0.3 M sodium acetate. The tube was incubated in a shaker overnight at 37 °C. The supernatant was transferred into a new tube and ethanol precipitated. The labelled DNA was dissolved in 25 µl 10 mM Tris-HCl, pH 8.8, 0.1 mM EDTA.

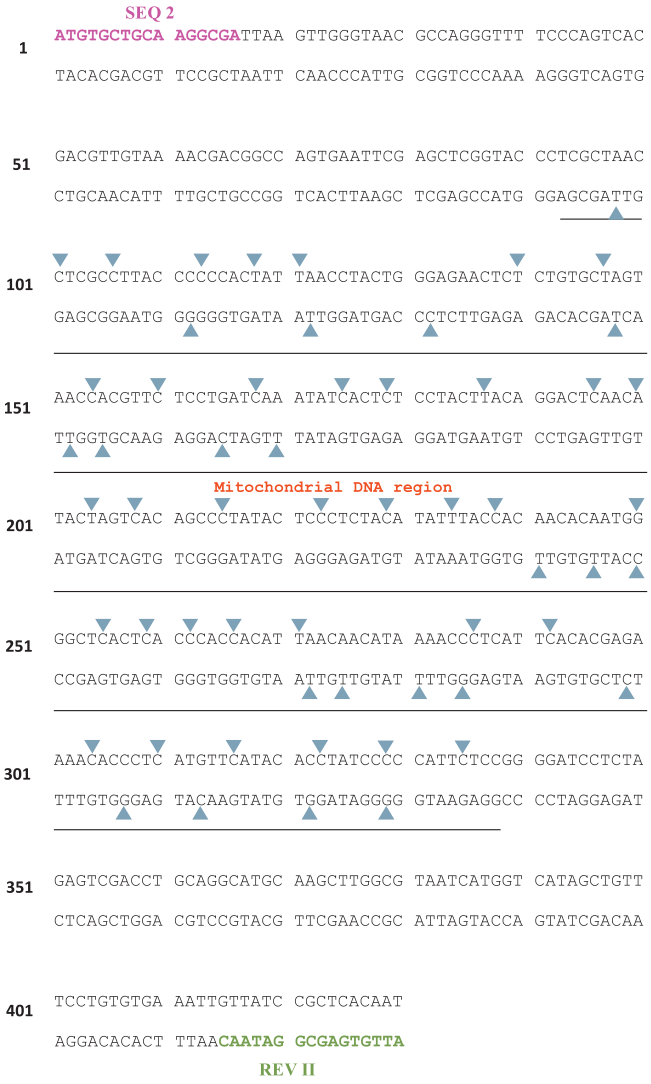

Fig. 1. The sequence of Mito 15 and UV damage sites.
The mitochondrial DNA insert is indicated by the black line and corresponds to bp 11,851–12,097 in the hg19 human mitochondrial sequence. The Seq2 (crimson) and the Rev2 (green) oligonucleotides were used as PCR primers. Using Seq2 and Rev2 as primers, the PCR product is 430 bp in length. The blue arrows indicate the UV damage sites detected from end-labelling and the linear amplification procedures; the arrow depicts either a UV breakage site or an adduct stop site or both. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.2. 3′-End-labelling

The 3′-fluorescent end-labelling procedure was performed as previously described [44]. Mitochondrial PCR products were obtained with the oligonucleotides 5′-TGTGCTGCAAGGCGA-3′ (Seq3) and Rev2 as primers for the top strand; and 5′-TTGTGAGCGCGGATAAC-3′ (Rev3) and Seq2 as primers for the bottom strand. The thermal cycling conditions were performed as described above. The PCR products were subjected to treatment with exonuclease I (New England Biolabs) and shrimp alkaline phosphatase (New England Biolabs) (Exo-SAP) to eliminate excess primers and dNTPs in the PCR reaction. A 12 µl reaction comprised 10 µl of the PCR mixture, 0.1 µl of 20 U/µl exonuclease I, 0.1 µl of 1 U/µl of shrimp alkaline phosphatase, 0.2 µl of 10 × exonuclease I buffer and 1.6 µl Milli-Q H_2O. The mixture of reaction was then incubated at 37 °C for 30 min, followed by enzyme inactivation at 80 °C for 15 min. The Exo-SAP treated PCR products were then placed in the 3′-FAM labelling reaction. In a total volume of 25 µl, there